THE EFFECT OF COLONY VERSUS ISOLATED HOUSING ON THE SELF ADMINISTRATION OF ALCOHOL AND RISK-TAKING BEHAVIORS IN AN ALCOHOL- PREFERRING RAT MODEL

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ABSTRACT

Alcohol is a psychoactive substance and may be the first recreational chemical produced by humans (Doweiko & Evans, 2019, p.45). Alcohol, when consumed chronically throughout the lifespan, has been shown to be damaging to the brain and internal organs (Novier et al., 2015; National Cancer Institute, 2020). Stress has been shown to be a major obstacle for alcohol rehabilitation and increases the risk of alcohol relapse (Pang et al., 2019). One form of stress commonly experienced by those affected by alcohol use disorder is social isolation, which has been shown to increase alcohol consumption in previous research using rodent models (Johnson et al., 2015; Lopez et al., 2011). This study evaluated the effectiveness of social enrichment in decreasing the average consumption of ethanol in alcohol-preferring female rats and how it can mediate risk-taking behavior.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

INTRODUCTION

Alcohol is one of the most commonly used psychoactive substances in the United States. In 2018, 86.3 percent of people ages 18 or older reported that they drank alcohol at some point in their lifetime (National Institute of Alcohol Abuse and Alcoholism, 2020). Following the consumption of alcohol, adult humans begin to show deficits in cognitive tasks such as those assessing semantic memory, verbal fluency, information-processing, working memory, and attention and planning (Novier et al., 2015). In addition to cognitive deficits, the consumption of alcohol has been associated with several cancers including mouth, esophagus, pharynx, larynx, liver, colon, and rectum in men and women. These risks increase after one drink daily for women and two drinks daily for men (National Cancer Institute, 2020).

Stress has been shown to be a major obstacle for alcohol rehabilitation and increases the risk of alcohol relapse (Pang et al., 2019). Anxiety disorders are also strongly associated with alcohol dependence (Smith & Randall, 2012). Alcoholism can lead to a variety of biopsychosocial problems and, as a result, anxiety can arise from disturbances in these areas (Smith & Randall, 2012). Individuals are more likely to endorse self-medication with alcohol alone with the highest rates being in those with general anxiety disorder (Smith & Randall, 2012).

Social Isolation as a Form of Stress

Addiction itself is a socially isolating disease and social support is an important element of treatment planning (Johnson et al., 2015). The relationship between social interaction and stress can be quite complicated; the social environment can mediate

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stressful situations, it can be a cause of stress, or stress can affect subsequent social behavior (Beery & Kaufer, 2015). Social isolation as a stressor in rodents has been associated with alterations in behavior including aggression, mating behavior, learning, and pain sensitivity, with these alterations being more severe in females than in males (Beery & Kaufer, 2015).

Adolescents who do not report having close friendships consistently have lower levels of self-esteem and more psychological symptoms of maladjustment (Hall-Lande et al., 2007). Additionally, adolescents who report a lack of social support and feelings of isolation may engage in self-harm, such as suicidal ideation and suicide attempts (Hall-Lande et al., 2007). In studies regarding post-weaning social isolation in rodents, male and female rats that were reared in isolation from pre- to mid-adolescence exhibited increased latency to emerge into an unfamiliar open-field, decreased center entries, and decreased defensive burying at postnatal day 40 (Lukkes et al., 2009). The limited number of studies that have used female rats precludes similar comparisons, but one common finding is that isolation rearing and/or social deprivation appears to increase anxiety-like behavior in female rats regardless of onset of isolation, but more research needs to be done in post-weaning social isolation in females rats (Lukkes et al., 2009).

Social Isolation and Alcohol Consumption

Social isolation and social connection are important influences on youth behavior that shape a host of outcomes including substance abuse (Johnson et al., 2015). In a study done by Evans and colleagues (2019), which examined the effects of social isolation on anxiety-like behavior and alcohol binge drinking in alcohol-preferring mice, they found the effects of social isolation were sex- and concentration-dependent. In male mice,

isolation increased intake in 20% and 30% ethanol concentrations, but in female mice, an increased intake of 30% ethanol concentration was seen in socialized animals (Evans et al., 2019). In mice that were isolated for 40 days immediately after weaning, males and females that were socially isolated consumed significantly more ethanol than mice that were group-housed (Lopez et al., 2011). These results may implicate a major gender gap in how social isolation affects men and women differently, as well as that differences differ case by case.

One well-known cause of social isolation, in the form of social distancing, for the past year was the spread of the COVID-19 virus (Hwang et al., 2020). The series of lockdowns associated with COVID-19 not only introduced social isolation as a stressor, but also loss of employment, loss of loved ones, working from home, management of children's schooling, and constrained financial resources (Calina et al., 2021). As a result from the COVID-19 pandemic lockdowns, a cross-sectional study done in China revealed an increased daily usage of alcohol in 32% of daily drinkers (Sharma et al., 2020).

Alcohol, Anxiety, and Risk-Taking Behavior

Alcohol consumption increases risk-taking behaviors such as aggression, drunk-driving, and risky sexual encounters (Erskine-Shaw et al., 2017). Adolescents show increases in risk-taking and sensation-seeking, which may include the frequent initiation of alcohol and other drug use, and elevated levels of alcohol consumption relative to those of adults (Spear, 2018).

Anxiety also has an effect on risk-taking and typically the more anxious an individual is, the less risks he or she takes (Giorgetta et al., 2012). Opposingly, some researchers have found that animals exposed to stress, through the exposure to predator

odor or raised platforms, had increases in risk-taking behaviors and novelty-seeking behaviors (Toledo-Rodriguez & Sandi, 2011). Animals exposed to thes stressors (predator odor and placement on an elevated platform) had increased time spent in the open arms of the Elevated Plus Maze (EPM), time spent head-dipping, and number of entries into the EPM open arms.

Purpose/Hypotheses

The purpose of this project was to evaluate whether colony housing would decrease the amount of ethanol consumed by alcohol-preferring rats. It was hypothesized that as a result of the decreased alcohol intake, the rats in colony housing would engage in less risk-taking behaviors than those in isolated housing. It was hypothesized that there would be a significant difference in ethanol consumption and risk-taking behaviors between the rats that switch housing conditions and the rats that remain in the same housing condition. Animals that have been switched from isolated to colony housing would consume more ethanol than animals switched from colony to isolated housing. The goal of this experiment was to inform clinical models of the role of social enrichment in the treatment of alcoholism.

METHODS

Animals and Housing

Thirty-eight Wistar Han® RCC female rats of 3 weeks old, postnatal day 21, were ordered from Envigo and transported to the Jackson Street Laboratory. Rats were either housed isolated (one to a cage) in a semi-self-contained housing system (Optirat©) or housed in a colony setting in a plywood box (4ftx8ftx2ft). All rats, regardless of housing condition, were given standard rat chow and hard-wood (sani-chip) bedding. The vivarium was maintained on a 12/12-hour light-dark cycle. Six of the Wistar Han® rats were used as strictly control animals and were housed in pairs. These 6 rats were used as a control for the risk-taking behavioral tests. Subjects assigned to colony housing were marked for identification using a nontoxic marker. A total of 38 subjects were utilized in this project with one attritional loss. Group assignments were as follows:

- Group 1: Isolated Housing only (8)
- Group 2: Colony Housing only (8)
- Group 3: Isolated to Colony Housing (8)
- Group 4: Colony to Isolated Housing (7)
- Group 5: Control (6)

Through various testing, Wistar Han® RCC rats have been proven to be a good model for toxicology, oncology, teratology, aging, and general purpose (Envigo, 2020). In general, laboratory animals do not readily drink alcohol and in most of the cases their consumption is too low to pharmacologically manipulate alcohol drinking or to evaluate the neurobiological consequences of alcohol exposure (Ciccocioppo, 2013). Therefore, using the alcohol-preferring Wistar Han® RCC rat would provide the best model for

studying alcoholism. Additionally, the vast majority of long-term post-weaning isolation studies utilize male rats, therefore using strictly female rats will be contributing to research in this area, not to mention it eliminated breeding within the colony housing condition (Lukkes,et al., 2009). Rats in the adolescent age range have been shown to experience more cognitive impairment than in adult rats and the adolescent brain displays higher sensitivity to alcohol-induced brain damage (Spanagel et al., 2014).

Materials

Housing Condition

In order to reduce stress and potential stress-related experimental confounds, all animals were given a period of three days to acclimate to the lab conditions (University of Kentucky Research, 2020). During the acclimation period, all animals were handled daily and were housed in pairs in the vivarium. After acclimation, animals were randomly assigned to either colony or isolated housing. The colony housing condition consisted of half of the animals in a 4ft x 8ft x 2ft plywood enclosure where they had free access to water, food, and enrichment in the form of PVC pipe tunnels, teething bones, and enrichment squares. **(Figure 1).**

Figure 1 - Enclosure

Note. Colony-housed animals were placed into a 4ft by 8ft by 2ft enclosure which was equipped with metal railings for food and water/ethanol dispensers to rest on.

The isolated housing condition consisted of half of the animals individually housed with free access to food and water. Finally, the control group consisted of 6 animals housed in pairs of 2 with free access to food and water throughout the study.

Ethanol Administration

Ethanol self-administration was conducted using a free-access, two-bottle choice design meaning that during the protocol, all rats (excluding the control) had access to both an ethanol solution and regular tap water, regardless of housing condition. This method is non-invasive and presents face and construct validity as a model of human alcohol consumption (Planeta, 2013). During the 20 day protocol, the rats were exposed to 4 different alcohol concentrations for 5 days each starting with a 5% ethanol solution (v/v) and ending with a 20% ethanol solution. Ethanol intake was measured by weighing water and ethanol bottles once every 24 hours (Planeta, 2013). The position of the water and ethanol bottles in the cage were switched every 5 days to avoid the development of place preference. Gradually increasing the concentration of ethanol controlled for tolerance. Compared to non-preferring animals, alcohol-preferring rats are less sensitive to the sedative-hypnotic effects of ethanol and develop tolerance more quickly to high-dose ethanol (Mcbride & Li, 1998). Additionally, adolescent rats tend to increase their alcohol consumption following stress, in this case social isolation, in a delayed manner (Spanagel et al., 2014). Therefore, 5 days for each concentration accommodates the delayed increase in consumption.

Behavioral Measures

Open Field Test (OFT)

The OFT is designed to measure anxiety-like behavior in rodents (Seibenhener & Wooten, 2015). Anxiety-like behaviors have been shown to be related to the avoidance of risk, therefore the more time spent in the center of the OF would indicate a reduction in anxiety and, as a result, a reduction in risk aversion (Charpentier et al., 2017). Each rat was placed gently into a light grey novel box measuring 60cm by 60cm and was allowed to explore for 5 minutes. Animals were placed in the apparatus facing the wall and activity was recorded through hand-scoring using stopwatches. The center and surround were marked with a permanent marker for scoring purposes. All sessions were video recorded in order to revisit the trials as needed. Data collected included: latency(s) to first enter the center of the OFT, time(s) spent in the perimeter, and time spent in the center. Experimental animals were transported into the testing room 30 minutes prior to the beginning of the first trial to allow for habituation to the condition of the behavioral testing room (Seibenhener & Wooten, 2015). The open field arena was wiped down with a 70% ethanol solution before testing and between each animal to remove any scent cues left by the previous rodent.

Elevated Zero Maze (EZM)

The EZM is a test that measures anxiety and risk-taking behavior. The EZM is a good measure of risk-taking because the entries and percentage of time spent in the open arms is an example of novelty seeking, which can be seen as a reduction of stress (Buckman et al., 2009) The EZM consists of a raised “O” shaped platform divided into two sections with surrounding edges and two sections with no surrounding edges. The

EZM forces the animal to be in either an open arm or a closed arm because it does not have a middle intersection. Risk-taking behavior was measured by recording the number of alterations made between the two closed arm sections, the time spent in the open arms, the time spent in the closed arms, the latency for the animal to leave the first closed arm, and the number of head dips. Being in a portion of the maze versus the other was operationally defined as having all four paws in the area. Head dips were operationally defined as the animal's nose going completely over the edge of the maze. Alterations were operationally defined as the animal going from one closed arm segment to the next closed arm segment. Experimental animals were transported into the testing room 30 minutes prior to the beginning of the first trial to allow for habituation to the condition of the behavioral testing room (Komada et al., 2008). The EZM was wiped down with a 70% ethanol solution before testing and between each animal to remove any scent cues left by the previous rodent.

Procedures

Upon the rodents' arrival, they had 3 days of acclimation to the lab environment. After the acclimation period, the rats were randomly assigned to isolated housing (16), colony housing (16), or control housing (6). In these two weeks, they had free access to food and water. After two weeks in the rodents' initial housing condition, half of each housing condition was randomly selected to switch to the opposing condition. Animals stayed in these conditions for an additional 2 weeks. On day 28 (after beginning of housing protocol), ethanol solutions were placed into both conditions, replacing half of the water bottles. On days 28-32, the rats had free access to 5% ethanol solution (v/v). On days 33-37, the ethanol bottles were replaced with a 10% ethanol solution (v/v). On days

38-42, the ethanol bottles were replaced with a 15% ethanol solution. Finally, on days 43-47, the ethanol bottles were replaced with a 20% ethanol solution. After the 20 day alcohol protocol, all ethanol solutions were removed from all cages and the enclosure. After a three day rest period, the rats began behavioral tests assessing risk-taking beginning with the OFT and ending with the EZM. The OFT was a one day protocol with each rat getting a single 5 minute trial. The EZM was also a one day protocol with each rat getting a single 5 minute trial. After all behavior tests were completed, 31 rats were euthanized according to federal guidelines and the rest (the 6 control animals) were set aside to be repurposed for other studies.

Statistical analysis

A mixed ANOVA was conducted to analyze consumption across the weeks; days were the within-subjects factor and group assignment were the between-subjects factor. A one-way ANOVA was utilized for dependent variables measured in the OF and EZM tests. All data was analyzed through the use of Jamovi and SPSS Version 25 software.

RESULTS

Ethanol Consumption

A mixed ANOVA was conducted on the 5-day averages of ethanol consumption to assess differences between housing condition groups. There was a significant within-subjects effect on weekly ethanol consumption, F (1.97, 31.50) = 75.7, $p < 0.001$. These results indicate that as days went on and ethanol concentrations went up, all rats consumed less ethanol. **(Table 1)**.

Table 1 - Ethanol Concentration

Estimated Marginal Means - Ethanol Concentration

			95% Confidence Interval	
Ethanol Concentration	**Mean**	**SE**	**Lower**	**Upper**
5%	38.03	1.83	34.37	41.69
10%	10.32	1.83	6.66	13.98
15%	7.43	1.83	3.77	11.09
20%	5.52	1.83	1.86	9.18

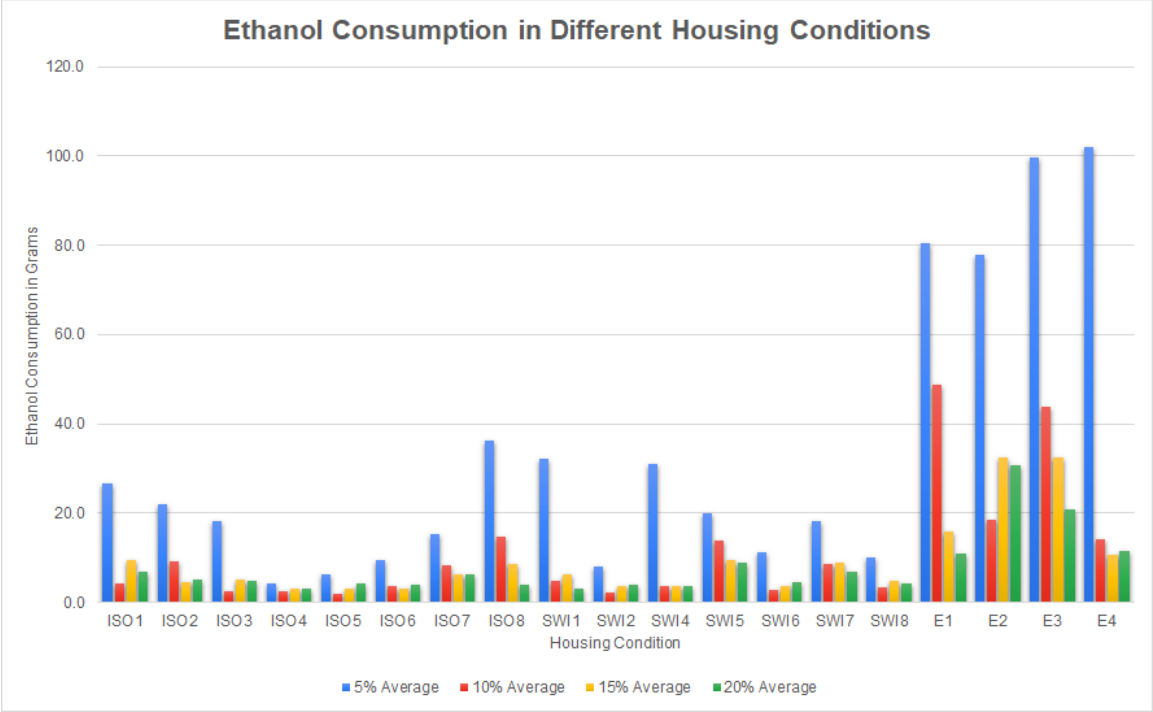
There was also a significant between-subject effect in consumption between housing condition groups, F (2, 16) = 82.2, $p < 0.001$. These results indicate that over-all, across the 20 days of ethanol consumptions, the groups in the enclosure consumed significantly more than those in isolation. **(Table 2) (Figure 2).**

Table 2 – Housing Condition

Estimated Marginal Means - Housing Condition

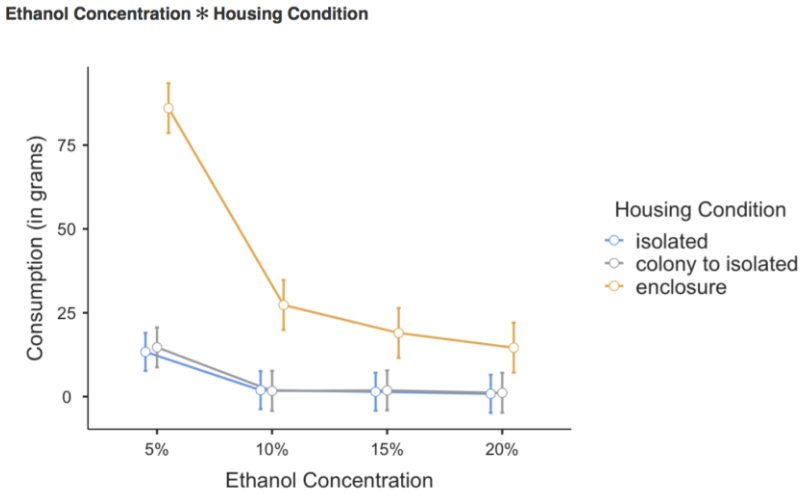
			95% Confidence Interval	
Housing Condition	**Mean**	**SE**	**Lower**	**Upper**
isolated	4.39	1.74	0.707	8.07
colony to isolated	4.84	1.77	1.092	8.59
enclosure	36.74	1.96	32.589	40.90

Figure 2 – Ethanol Consumption in Different Housing Conditions



There was also a significant interaction between group and ethanol concentration, F (3.94, 31.50) = 21.6, $p < 0.001$. **(Figure 3).** There were significant differences between housing conditions across the different ethanol concentrations.

*Figure 3 – Ethanol Concentration * Housing Condition*



Open Field Test

There was no significant differences in housing condition group on any risk-taking measures of the OFT; latency to enter the center, F (4, 14.2) = 2.13, p = 0.131, time spent in the perimeter, F (4, 15.8) = 1.90, p = 0.16, and time spent in the center, F (4, 15.9) = 2.02, p = 0.141.

Elevated Zero Maze

There were no significant differences in housing condition group on any risk-taking measures of the EZM; latency to leave the first closed arm, F (4, 14.2) = 1.403, p = 0.283, time spent in the open arms, F (4, 14.9) = 0.849, p = 0.516, time spent in the closed arms, F (4, 14.9) = 0.841, p = 0.521, number of head dips in the elevated zero maze, F (4, 14.7) = 1.61, p = 0.224, number of alterations made between closed arm segments, F (4, 15.4) = 1.573, p = 0.231.

DISCUSSION

The purpose of this study was to examine the effect of colony housing on the voluntary consumption of ethanol and risk-taking behavior in alcohol-preferring rats in their adolescent period. The study did not find any effect of housing condition on any of the risk-taking behavioral tests. The study did find an effect of housing condition on the consumption of ethanol, but the results were not in line with the initial hypotheses.

Experimental Methods

Ethanol Consumption

There were significant differences in ethanol consumption amongst housing conditions, but the results do not follow previous literature or support the predicted hypotheses. Results from the study revealed that rodents in the enclosure consumed more ethanol than rodents in strictly isolated conditions and rodents that switched from the enclosure to isolated conditions at 5%, 10%, and 15% ethanol concentration (v/v). Previous studies examining the effects of social isolation on ethanol consumption in rodents has focused on strictly male rodents or gender differences between males and females. Additionally, the limited amount of literature suggests and gives very different results from one another in terms of how social isolation affects women. Evans and colleagues (2019) found that the effects of social isolation alcohol-preferring mice were sex-dependent and socialized female mice consumed significantly more ethanol than isolated mice.

Open Field Test and Elevated Zero Maze

The results from the open field test and elevated zero maze did not support the predicted hypotheses. These results are not in line with a majority of previous literature

looking at the relationship between anxiety and risk-taking behavior. Previous research suggests that compared to socialized animals, isolated mice exhibit more anxiety-like behaviors (Evans et al., 2019). There is also literature that supports the findings of the current study. Toledo-Rodriguez and Sandi (2011) found that male and female rats who were exposed to predator urine and raised platforms had increases in risk-taking behaviors. Although not significant, the rats in the colony enclosure spent more time in the perimeter than the socially isolated animals. A significant difference may have been observed if larger sample sizes were utilized.

Limitations

One of the limitations of this study was the set-up of the enclosure. The enclosure was custom built by the researcher for this experiment. With the set-up of the enclosure, there was no way to compare the ethanol consumption of the colony-to-isolated and isolated-to-colony group. If a divider would have been placed in the enclosure to separate the colony and isolated-to-colony groups, the researcher would have been able to draw more comparisons. The researcher was also unable to track the individual ethanol consumption within the enclosure. If there would have been access to technology that would track and record the daily consumption of each individual animal, the researcher would have been able to examine the differences between animals within the enclosure.

Another limitation of the study was the sample size of the groups. Due to the set up of the enclosure, the number of groups went from 5 to 3: enclosure, isolated, and colony-to-isolated. With a larger sample size, there is a possibility that it would have provided more significant results and greater power. Larger sample sizes could have also provided more significant results in the open field test and elevated zero maze.

Future Directions

Future research should examine the effect of colony housing in other areas of research. The research that is available on colony housing is typically done using chronic subordinate colony housing (CSC), which has been established as a stress parameter. In the current study, the colony housing condition was utilized as a way to relieve stress.

Future studies could investigate the use of colony housing with involuntary administration of ethanol through injections or vapor. These two forms of ethanol administration allow researchers to control for differences in amount consumed and ensure that each animal is achieving a specific blood alcohol concentration (BAC).

Future studies could investigate the effect of colony housing on stress in the form of chronic variable stress. Similarly to that of Evans and colleagues (2019), researchers could examine how social enrichment alleviates negative effects caused by the exposure of several stressors. Additionally, future researchers could examine if the time of intervention plays any role in its effectiveness.

CONCLUSION

Alcohol has been shown to cause negative physiological and cognitive effects when consumed chronically. The findings in the current study found that housing does have an effect on ethanol consumption, but not on risk-taking behavior. Social housing was found to increase ethanol intake in alcohol-preferring female rats. These results both did and did not align with previous studies examining how social isolation affects ethanol consumption in rats and humans. Further research needs to be conducted on how isolation affects women in terms of substance abuse in order to develop more specific treatment plans catered towards women.

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APPENDIX

IACUC APPROVAL

ANGELO STATE UNIVERSITY

College of Graduate Studies & Research

Institutional Animal Care & Use Committee

January 4, 2021

Dr. Steven Brewer, Assistant Professor
Department of Psychology and Sociology
Angelo State University
San Angelo, TX 76909

Your proposed project titled, "THE EFFECT OF COLONY VERSUS ISOLATED HOUSING ON THE SELF ADMINISTRATION OF ALCOHOL AND RISK TAKING BEHAVIORS IN AN ALCOHOL-PREFERRING RAT MODEL" was reviewed by Angelo State University's Institutional Animal Care and Use Committee (IACUC) in accordance with the regulations set forth in the Animal Welfare Act and P.L. 99-158.

This protocol was approved for three years, effective January 4, 2021 and it expires three years from this date; however, an annual review and progress report form (www.angelo.edu/content/files/22583-iacuc-annual-review-progressreport) for this project is due on August 15 of each year. If the study will continue beyond three years, you must submit a request for continuation before the current protocol expires.

The protocol number for your approved project is 2020-106. Please include this number in the subject line of in all future communications with the IACUC regarding the protocol.

Sincerely,

Chase Runyan, Ph.D.
Co-Chair, Institutional Animal Care and Use Committee

BIOGRAPHY

Hali Rianne Johns graduated from Angelo State University with a Bachelor of Science degree in 2018. She majored in psychology and minored in mathematics. Hali Rianne Johns was a member of Psychology Club and briefly held a position as acting secretary for the organization. She is also a member of the Texas Zeta Chapter of Pi Mu Epsilon, a national mathematics honor society. Hali worked at Angelo State University as a lab assistant and graduate assistant in the Department of Psychology and Sociology. In the past two years, Hali has worked on six research projects and presented once at the Graduate Symposium at Angelo State University. Hali Rianne Johns plans to continue studying and conducting research on the effects of alcohol and other psychoactive substances after obtaining her master's degree.